

Conformational Plasticity Revealed by the Co-crystal Structure of the C-type Lectin-like Receptor NKG2D and its Class I MHC-like Ligand ULBP3

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Beamline(s): X9B

Introduction: NKG2D, a member of CTLR superfamily and distantly related to NKG2A, B, C, and E, is found on NK cells to trigger cytotoxicity against certain tumor cells, on CD8⁺ $\alpha\beta$ T and $\gamma\delta$ T cells to provide a co-stimulatory signal against virally infected cells. Efforts in searching for NKG2D ligands have led to the identification of a group of human cytomegalovirus glycoproteins UL16 binding proteins, named as ULBPs as ligands to human NKG2D. Here, we report the 2.6 Å resolution structure of the extracellular domain of human NKG2D and ULBP3 complex. Comparison of this complex with previous NKG2D structures reveals the molecular basis of the broad specificity of NKG2D. The crystal structure of ULBP3 provides the first image of a class I-like molecule that lacks the $\alpha 3$ domain and is GPI-anchored to the membrane.

Methods and Materials: The crystals of NKG2D and ULBP3 complex were flash frozen at 100 K. X-ray diffraction data from single crystals were collected using an ADSC Quantum IV CCD detector at the X9B beam line of the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory and processed with HKL2000. The crystals belong to a space group $P4_32_12$ with cell dimensions $a=b=62.05$ and $c=237.3$ Å, contain one dimer of NKG2D and one ULBP3 per asymmetric unit, and diffract to 2.6 Å.

The NKG2D homodimer (PDB accession number 1HQ8) was used as a model to localize the receptor in the complex by molecular replacement using AmoRe. The NKG2D phased density map was insufficient to localize ULBP3. A SeMet MAD dataset was collected from the crystals containing SeMet-ULBP3 at the X9B beamline (NSLS). Six selenomethionines were found in the difference electron density map with phases from NKG2D using program CNS, version 1.0. After density modification including solvent flipping, the electron density of ULBP3 was traced unambiguously. Model adjustments and rebuilding were done using program O. The positional and individual B -factor refinement was carried out using a maximum likelihood target function of CNS v1.0. The refined model consists of residues 94-215 from NKG2D and 9-186 from ULBP3 with one loop of ULBP3, residues 90-96, missing.

Results: The structure of a human NKG2D receptor in complex with its ligand ULBP3 has been determined by a combination of molecular replacement and multiwavelength anomalous dispersion (MAD) methods and refined to 2.6 Å resolution. The refined (2Fo-Fc) electron density is continuous throughout the complex except for one surface loop of ULBP3, which is from residue 90 to 96 at the C-terminal end of the $\alpha 1$ -helix and is away from the receptor interface. The refined R-factors are 23.0% and 27.2% for R_{cryst} and R_{free} , respectively. Each crystallographic asymmetric unit contains one NKG2D dimer and one ULBP3 molecule.

The overall shape of the NKG2D/ULBP3 complex resembles a crab preying on a seashell (Figure 1). The crab shaped receptor uses its claw shaped β -strands and loops, at the end opposite to the N- and C- termini, to grab on the ridge-shaped helical surface of ULBP3. This mode of complex formation is also observed in NKG2D/MICA complex. The relative orientation between NKG2D and ULBP3 is similar to that between KIR and HLA, and between TCR and their MHC ligands. The long axis of the receptor is diagonally across the helical axis of ULBP3. The receptor footprints the C-terminal half of $\alpha 1$ -helix and the N-terminal half of the $\alpha 3$ -helix of ULBP3. Both subunits of the NKG2D bind ULBP3 with identical receptor loops. The interaction between a homodimeric NKG2D and the asymmetrical ULBP3, results in an asymmetrical receptor subunit orientation different from that observed in the dyad related ligand-free murine receptor.

Conclusions: NKG2D is known to trigger the NK cells lysis of various tumor and virally infected cells. In the NKG2D/ULBP3 complex, the structure of ULBP3 resembles that of the $\alpha 1$ and $\alpha 2$ domains of classical MHC molecules without a bound peptide. The lack of $\alpha 3$ and $\beta 2m$ domains is compensated by replacing two hydrophobic patches at the underside of class I MHC β -sheet floor with a group of hydrophilic and charged residues in ULBP3. NKG2D binds diagonally across the ULBP3 α -helices, creating a complementary interface, an asymmetrical subunit orientation and local conformational adjustments in the receptor. The interface is stabilized primarily by hydrogen bonds and hydrophobic interactions. Unlike the KIR receptors that recognize a conserved HLA region by a lock-and-key mechanism, NKG2D recognizes diverse ligands by an induced-fit mechanism.

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Ribbon drawing of the NKG2D-ULBP3 complex.